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Clinical Radiology

The Importance of Post Neoadjuvant Histological Assessment and Need for Standardisation

--Manuscript Draft--

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Abstract:	Neoadjuvant therapy is increasingly being recognised as a management option for patients with primary invasive breast carcinoma; this may take the form of primary endocrine treatment or primary chemotherapy. Surgical specimens from women treated with neoadjuvant treatments, particularly primary chemotherapy, may cause challenges for the histopathologist in handling and interpretation and have, in the past, been sampled, evaluated and reported in a non-standardised way. This limits comparison between clinical trials and potentially provides clinicians and patients with sub-optimal prognostic information. We describe here some of the difficulties faced and the recommendations and standards now applied.

Invited Review:

**The Importance of Post Neoadjuvant Histological Assessment and Need for
Standardisation**

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Both authors contributed to the preparation and editing of this manuscript and have no conflicts of interest to declare.

Revised Version of 'The Importance of Post Neoadjuvant Histological
Assessment and Need for Standardisation'

We would like to submit our revised review article entitled 'The Importance of Post Neoadjuvant Histological Assessment and Need for Standardisation' for your consideration. We thank the reviewers for their constructive comments, and are glad that they found the review interesting, stimulating and informative.

In response to Reviewer 1's comment:

1. 'Clips should be sought by x-raying not only macroscopically. This should be added and the value of specimen x-raying addressed.'

We thank the reviewer for this comment. We do mention under the section entitled 'Macroscopy and Microscopy' that 'access to pre- and post-treatment radiological images and reports are invaluable.' Of course, in difficult cases where there has been a very good response to neoadjuvant therapies, it may be difficult to macroscopically identify the tumour bed and/or clip(s). In such cases, specimen (and, very rarely, specimen slice) X-ray may be of benefit, but this is very uncommonly required and therefore not used routinely (as the 'MRI coil' can be seen on slicing the specimen). Indeed, as stated in the RCPATH guidelines: 'Often the marker can be detected macroscopically on thin slicing of the specimen'. We have, however, added comment that this may be helpful in rare cases.

2. 'Rate of response to dual anti her2 therapy is much higher. Recent reports show over 70 percent pCR'.

We thank the reviewer for this observation but would note that the rates of pCR in the neoadjuvant setting of trials in the *peer-reviewed* published literature for non-metastatic breast cancer are generally just over 50% with dual agent anti-HER2 therapy (typically peer-reviewed data is available for

trastuzumab and lapatanib; e.g. in NeoALTTO, CALGB-40601/II and in NSABP-B41/III). Although presentations have reported higher rates with pertuzumab and trastuzumab, we have not been able to source these in peer-reviewed form. The papers we have referenced in relation to response rates with anti-Her2 therapy include a recent meta-analysis, which includes 36 studies (5768 patients). To our knowledge, this is the most recent large-scale review of the available published data. We have not, therefore, amended this section, other than to add brief comment about a dual anti-HER2 approach.

3. 'There are some important references that are worth including and referring to.'

It is unclear if the reviewer if the reviewer here is referring to the RCPATH pathology guidelines (see below) or if there are additional references that they feel we should include. We would be happy to re-edit if these are specified.

4. 'The authors should highlight what guidelines are available. Refer to Pinder et al 2014 paper of neo adjuvant reporting, and RCPATH guidelines on this area.'

We thank the reviewer for drawing our attention to this oversight. We have added in the suggested references.

5. 'Please provide some imaging photos: e.g. clip site, calcifications, imaging before and after treatment to be of interest to a radiology readership. You may also wish to address relevant radiological issues such as calcification in this context ...etc.'

In order to keep to within our expertise (which is not radiological!), and also within the word count, we have kept specifically to a brief overview of clinical details and concentrated on the histopathological examination and assessment; in particular we suspect that a review on standardisation of the neoadjuvant therapy radiologically is itself more than a single review article.

For this reason we have almost completely steered away from issues of mammography assessment, peri-treatment radiological assessment (when, by MRI or US), re-sampling of radiological calcifications, CT, MRI assessment etc etc and we have avoided impudently including any radiological images. If the editor feels strongly such images would add to the document we would be happy to include these, but would also then need to add significantly to the word count to explain why they had been incorporated and the issues of controversy?

In response to Reviewer 3's comments:

'This is an excellent and timely article on a very important subject. The article is thorough and presents the information available in a clear and logical way which makes it easy for the reader to understand. Well referenced, good illustrations which also add to the quality of the paper. I suggest including a table with more detail on RCB categories.'

We thank the reviewer for this useful suggestion. A table has been included.

We hope that you find the revised version satisfactory. Please do not hesitate to contact me should you require any further information.

Abstract

Neoadjuvant therapy is increasingly being recognised as a management option for patients with primary invasive breast carcinoma; this may take the form of primary endocrine treatment or primary chemotherapy. Surgical specimens from women treated with neoadjuvant treatments, particularly primary chemotherapy, may cause challenges for the histopathologist in handling and interpretation and have, in the past, been sampled, evaluated and reported in a non-standardised way. This limits comparison between clinical trials and potentially provides clinicians and patients with sub-optimal prognostic information. We describe here some of the difficulties faced and the recommendations and standards now applied.

Abstract

Neoadjuvant therapy is increasingly being recognised as a management option for patients with primary invasive breast carcinoma; this may take the form of primary endocrine treatment or primary chemotherapy. Surgical specimens from women treated with neoadjuvant treatments, particularly primary chemotherapy, may cause challenges for the histopathologist in handling and interpretation and have, in the past, been sampled, evaluated and reported in a non-standardised way. This limits comparison between clinical trials and potentially provides clinicians and patients with sub-optimal prognostic information. We describe here some of the difficulties faced and the recommendations and standards now applied.

Introduction

The management of early breast cancer is constantly changing. As new treatments emerge, and as our understanding of the biology of the disease evolves, the use of neoadjuvant systemic therapies is increasing. Originally a means to manage patients with inoperable locally advanced breast cancer and subsequently to optimise cosmesis in patients with large tumours, neoadjuvant systemic therapy is now integral to the treatment of patients with early stage disease, even those with smaller lesions suitable for breast conservation at presentation. Large clinical trials have shown no differences in survival for patients given systemic therapy pre-, or post-, surgery (1-3).

Accurately assessing the efficacy of such treatments in the surgical excision specimen is important, not only for understanding which patients will develop recurrent or metastatic disease (i.e. prognostication), but also in uncovering mechanisms of resistance that facilitate these poor outcomes. However, standardising assessments between laboratories across the globe has proven challenging.

Pathological Complete Response

It is well established that patients with tumours that have undergone complete pathological response (pCR) have a better prognosis than those that have not. In 2012, an 'International Consensus Conference on the Current Status and Future of Neoadjuvant Systemic Therapy in

Primary Breast Cancer' (4) recommended that a complete pathological response (pCR) should be histologically determined, and should be defined as an absence of invasive cancer both in the breast and axillary lymph nodes. This definition was also recommended by the Residual Disease Characterization Working Group of the Breast International Group-North American Breast Cancer Group (BIG-NABCG) collaboration in 2015 (5). This definition may seem obvious, but others have historically been used by various groups; pCR has previously been applied to refer to an absence of disease in breast alone (i.e. with either uninvolved or metastatic disease in the axilla) or for cases with no carcinoma in either the breast or the axilla, and has even included a range from complete absence of any malignancy in the breast through to a definition including cases with minimal residual invasive disease.

The significance of residual disease components in the breast tissue is now clearer. Although some have reported a more adverse event-free survival in patients with residual ductal carcinoma in situ (DCIS), almost none have identified an effect on overall survival when compared to those with no in situ or invasive carcinoma after neoadjuvant chemotherapy (6). For this reason, residual DCIS in the breast should be documented, but is considered for prognostication purposes as pCR. Conversely, residual 'pure' lympho-vascular invasion, even in lymph node negative patients, is a poor prognostic feature and should not be considered a complete response (7).

Specifically, the definition of pCR that is now recommended includes an absence of disease in both breast and axilla. Although typically both axillary and breast foci respond in a similar fashion, it is not uncommon to have no residual disease in the breast but metastatic tumour foci remaining in the lymph nodes (approximately 5% of cases). Residual disease in the axilla, even

when the primary breast tumour has completely responded to neoadjuvant chemotherapy (NACT), confers a worse prognosis (8).

Selection of patients most suitable for neoadjuvant therapy is an important part of the pathologists' role and their contribution to the multidisciplinary team by providing accurate and timely interpretation of core biopsy grade (9) and receptor status (10). The highest pCR rates are seen in triple negative breast cancer (TNBC; up to 33.6% following anthracycline based chemotherapy) and HER2-positive disease (up to just over 50% after treatment with dual anti-HER2 targeted therapy) (11-13).

However, it is important to remember that, although high grade invasive cancers are those most likely to achieve pCR following NACT, patients with low grade tumours (that typically do not respond as markedly to NACT) still have a good outcome, since the tumour was more innocuous at baseline. Low grade invasive carcinoma is also likely to be oestrogen receptor (ER) positive and neoadjuvant endocrine therapy (NAET) may be a more appropriate option compared to grade 3 tumours where pCR is more likely with NACT (irrespective of immunohistochemical sub-type) (4, 14). ER-rich invasive lobular carcinoma, for example, typically responds relatively poorly to NACT but, in some series, a mean reduction in cancer volume of 66% has been reported with NAET (letrozole) (15). Interestingly, progesterone receptor (PR) negativity has been shown to be associated with higher rates of pCR to NACT in ER-positive HER2-negative breast cancer, but also with a worse long-term outcome in the same cohort of patients (16). Thus, frequency of pCR (generally reported at rates of 3-50%) is a reflection of thoroughness of histological sampling and examination (see below), clinico-pathological characteristics of the tumour and the type of therapy given. Despite best efforts

however, 60-85% of breast cancer patients typically have residual disease following neoadjuvant therapy at present (4). One important challenge, therefore, is to stratify the subset of patients who do not have pCR, both for prognostication and, potentially, to evaluate the efficacy of newer, ever- emerging therapies.

Macroscopy and Microscopy

It is imperative that pathologists examine and report specimens from patients who have received neoadjuvant systemic therapies in a standardised and thorough way (5, 17, 18). These specimens in particular require excellent multidisciplinary team working and provision of adequate, detailed clinical information on the histology request form. The sites of all of any multiple foci of carcinoma present prior to treatment should be examined microscopically and these may not be easily apparent in women who have had a good response. Diagrams are helpful and access to pre- and post-treatment radiological images and reports are invaluable. Markers inserted, even if the patient is advised to undergo mastectomy in some centres, and certainly for those undergoing breast-conserving surgery, are sought macroscopically and provide the nidus for histological examination. On rare occasions these may be difficult to see and then specimen slice x-ray can be performed. Residual tumours may be difficult to palpate post-neoadjuvant systemic therapy and are typically softer than pre- treatment. Often, all that is seen upon slicing is an ill-defined, pale area of stellate fibrosis. Occasionally, the tumour bed may look oedematous, with a glistening cut surface.

Tumours in patients in receipt of NACT more often undergo a scattered pattern of response,

compared to NAET where a central fibrotic area with a surrounding rim of residual invasive disease is common (19). However, not all ER positive tumours are equal, and it has been suggested that those with lower levels of ER expression (determined semi-quantitatively by, for example, Allred score) respond better to NACT, much like their triple negative counterparts, whilst those with high levels of ER expression typically respond to NAET. It is also worth noting that the duration of NAET required to induce the best response rates is invariably much longer than a course of NACT and that different pathological scoring systems have been used to quantify residual disease, precluding precise comparison of patterns of response to these different treatments.

Because of the scattered tumour response to NACT often seen, more extensive sampling is usually required than for samples from patients who have not received primary systemic therapy. Guidance recommends a universal approach to sampling that should not only include grossly visible tumour (if present) and the site of marker clip(s), but also the extent of the tumour bed/fibrosis and adjacent tissue to encompass the area of involvement by carcinoma before treatment (5). The largest cross-section of the pre-treatment area of involvement on slicing should be examined microscopically. If available, large 'mega-blocks' can be valuable but careful targeted and systematic examination is more appropriate than exhaustive sampling; some authorities recommend a minimum of one block per cm of pre-treatment tumour size, or at least 10 blocks in total, whichever is greater (20).

Microscopically, identifying residual disease can also be difficult, particularly where the response has been good (**Figure 1**). Where pCR has occurred, the tumour bed may consist only of fibrosis, which may be loose and oedematous or fibro-elastotic (**Figures 2 and 3**).

Typically, no breast epithelial structures are visible in such regions, providing both a useful diagnostic clue that this represents tumour bed and confirmation that the correct site has been sampled. Inflammation may be present to varying degrees and foamy macrophages may be abundant. In other cases, where treatment has been less effective, residual disease is clearly apparent, even though NACT may induce morphological changes such that the tumour may not resemble that in the original core biopsy, particularly if only a few cancer cells remain (**Figure 3**). Specifically, the component of histological grade may change; there may be fewer mitoses but a greater degree of tumour cell pleomorphism. Cancer cells may masquerade as macrophages and immunohistochemistry evaluation may be required to determine the nature of a scanty cell population.

Lymph nodes from patients receiving NACT should be sliced thinly, all embedded and haematoxylin and eosin-stained (H&E) sections assessed. Although immunohistochemistry for cytokeratin markers highlights cases with fibrosis with very scanty residual metastatic cells not seen on H&E stains, such 'occult' residual deposits do not seem to have prognostic value (21, 22) and 'routine' immunohistochemistry (rather than for the assessment of suspicious foci seen on H&E, which is recommended) is not deemed necessary. Nevertheless, isolated tumour cell clusters (ITCs) may be identified in a background of fibrous response to NACT and are not regarded as pCR but are interpreted as representing response of larger (micro- or macrometastatic) deposits to therapy given.

Assessment of Amount of Residual Disease

It seems inherently logical that the degree to which a tumour has responded to neoadjuvant treatment should reflect prognosis; thus patients with pCR have a better outcome than those with moderate response, who fare better than those with little tumour reaction to systemic therapy. However, how to classify this spectrum has proven elusive. A number of histological classification systems have been used to evaluate the degree of response to NACT (23-28), whilst few have been described to categorise response to NAET. The systems described have, however, been based on different cohorts of patients, with various chemotherapy regimens and have applied variable cut- offs for the scoring systems. There are few large-scale comparisons of the systems and local preference has largely been the basis for selecting one method over another. Nevertheless, conceptually, most of the methods described for classification of degree of response to NACT stratify therapeutic response (actually amount of residual disease) according to some or all of three criteria: (i) reduction of tumour size, (ii) reduction in cellularity of the tumour in the breast and (iii) the reduction in number and size of nodal metastases. Some compare the features of the tumour on the prior (pre-treatment) core biopsy, others simply assess tumour size and cellularity on the post- treatment histology specimen.

Controversy exists at present as to how best to record the size of the residual tumour post-NACT, regardless of location (i.e. breast or lymph node). The latest American Joint Committee on Cancer (AJCC) (TNM8) now recommend inclusion of only the largest contiguous area of tumour (tumour cells touching each other). In the breast, this poses a problem when the tumour responds with a scattered pattern, since individual foci may be separated from each other by intervening fibrosis. Thus, measuring the size of one focus alone would significantly underestimate the total volume of residual tumour. Similarly, the AJCC advocates a similar approach to lymph node deposits such that only the largest area in which cells are touching one another is measured. Clearly, for those nodes in which only disparate isolated tumour cell

clusters (ITC) remain, this is likely once again to underestimate the residual metastatic volume; in fact, such measurements would result in nodes bearing ITCs being classified as negative, when in fact, in the post-NACT setting, they are considered positive (i.e. not pCR). Indeed, there are no studies showing an association between this method of quantification and survival outcome at present. For this reason, we continue to measure the overall size of adjacent islands of metastatic disease that remains in the lymph nodes after NACT, unless clearly widely separated by normal nodal structure (i.e. not within a fibrous background that is in essence equivalent to the metastatic tumour bed) and to assess the overall extent of scattered foci of disease in the breast tissue.

For treatment naïve disease, the size of the nodal deposit is proportional to patient outcome, i.e. the smaller the metastasis, the less the effect on their prognosis. Even the way in which pathologists quantify and report disease in the axilla (i.e. isolated tumour cell clusters versus micro- and macro- metastasis) reflects the recognition that metastatic tumour volume is important (hence the afore- mentioned objections to the current AJCC recommendations). Whilst this holds true in the context of residual disease after NACT, it is also clear that response of the nodal metastasis significantly improves outcome; regression or reduction of nodal tumour volume improves disease-free survival and reduces risk of relapse (29). Patients with nodes showing features of prior node involvement, but with no residual tumour cells in the nodes have an intermediate prognosis between those with residual metastasis and those with normal negative lymph nodes (5, 28). Post NACT, pathologists should thus record not only the number of lymph nodes remaining positive, but whether or not any treatment-related changes (e.g. fibrosis) are present, the latter whether or not tumour cells are identified.

Rajan et al demonstrated that some tumours maintain a similar overall size after NACT to the pre-treatment core, but dramatically decrease in cellularity (30). This group went on to suggest that the product of these two measurements (i.e. overall remaining tumour size and tumour cellularity) was more clinically relevant in determining response than tumour size alone and to develop the Residual Cancer Burden (RCB) scoring system (28). This system is recommended by many as the 'preferred method for more detailed quantification of residual disease' (5). The major advantage of the RCB score is that it combines the extent of remaining tumour in the breast and its cellularity and size of nodal deposits in such a manner that it allows for both a continuous (numerical) and categorical output and has been shown to be associated significantly with long-term survival outcome. Mathematically, equal weight is given to the presence of residual disease in both the breast and the lymph nodes. The score is ultimately an assessment of overall tumour volume, with small changes in any of the parameters causing a change in the continuous score, and larger changes potentially causing a change in the RCB category (0 to III; **Table 1**). The lower the score, the lower the risk of distant recurrence. The score has been shown to be highly reproducible, even in the 'routine' diagnostic setting (31, 32). Other systems, such as the 6 grades of the Japanese Breast Cancer Society, have also been shown to be reproducible (33) but are nevertheless not widely utilised globally.

One of the limitations of all present systems for histological classification of degree of response to neoadjuvant systemic therapies is that they do not necessarily reflect the intrinsic biology of the tumour itself. There is scope, therefore, to refine this stratification method by incorporating biomarkers into the assessment. Indeed there is some evidence that inclusion of the proliferation marker, Ki67, to the RCB adds value and that a prognostic index incorporating RCB, post-treatment Ki67, grade and ER may provide even more prognostic information (34). However, in its present form the RCB has been shown to provide prognostic information across

the sub-types of invasive breast carcinoma (triple negative, HER2 positive and hormone receptor positive)(35) with estimates of 10- year relapse-free survival rates in the four RCB classes (RCB 0 (pCR), RCB I, RCB II, and RCB III) of 86%, 81%, 55% and 23% for triple negative, 83%, 97%, 74% and 52% for hormone receptor positive, and 95%, 77%, 47% and 21% for those patients with HER2 positive disease.

Other biomarkers are currently under investigation and are reported as potentially important as both predictors of likelihood of response to, and of prognostic value after, NACT. Whilst tumour-infiltrating lymphocytes (TILs) are reported to be of prognostic value in patients with triple negative breast cancer who have received NACT (as a marker of good prognosis, even in those patients with a large residual tumour burden) (36), further work is required on standardisation of this biomarker. Importantly, the infiltrating immune cells are likely to be functionally (and prognostically) variable depending on their location, nature of the lymphoid cells and the sub-type of the breast cancer. For example, in some series it is reported that lymphocyte predominant HER2 positive cancers are more likely to achieve pCR (37), whilst in other studies the levels of TILs in pre-treatment specimens has not been found to be significantly associated with response to NACT in this sub-type (38). Systematic review and meta-analyses indicates that TILs predict higher pCR rates in triple negative (OR = 2.49, 95% confidence intervals (CI) 1.61-3.83) and HER2 positive (OR = 5.05, 95% CI 2.86-8.92) breast cancers, but not in ER positive (OR = 6.21, 95%CI 0.86-45.15) disease (39); the situation is clearly complicated and not yet appropriate for routine reporting outwith the research setting.

What may, therefore, be more immediately relevant to pathologists is achieving consensus on the significance and value of 'simple' tumour biomarkers. Although some experts maintain that

reassessment of tumour receptors (e.g. ER, PR and HER2) on post-NACT residual tumour is essential (40), this is not universally recommended. It is clear that whilst some standardisation of handling and reporting specimens from patients receiving neoadjuvant treatments have been achieved, this is a continually evolving field and additional work is required.

Imaging Biomarkers

One ongoing area of research focus is the comparison of the results of various imaging modalities for assessment of treatment response (both during and after neoadjuvant systemic therapy) with pathological findings. One recent meta-analysis, which included 10 studies (six prospective and four retrospective), showed MRI to be more sensitive than FDG-PET/CT in evaluating response post-NACT (0.88 versus 0.57) but FDG-PET/CT to be more specific than MRI in evaluating response during therapy (0.69 versus 0.42) (37). The EUSOMA working group has for many years recommended MRI for the assessment of residual disease post-NACT (41) but the evidence on whether this is accurate across all sub-types of invasive cancer is less clear (42-45).

Additional imaging research is required to optimise methods for accurately categorising the amount of residual disease during neoadjuvant systemic treatment. Whilst at the end of treatment the correlation between the MRI and pathological measurements of residual tumour (i.e. in the excised specimen) ranges from 0.65 to 0.98, in some series only ~70% of MRI size evaluations were accurate (46). Series correlating standardised (end of treatment) RCB scores with imaging assessments will provide valuable data on the accuracy of the latter; this is likely to

be increasingly important for the accurate identification of women with significant residual tumour in planned clinical trials of further pre-operative systemic treatment. Clearly imaging biomarker studies of this type require correlation with standardised pathology categorisation and have been historically therefore been problematic, because of the lack of robust histopathological data.

Conclusion

The increased use of neoadjuvant systemic therapies for patients with invasive breast cancer, and the greater proportion of patients achieving good responses to more personalised treatments, has highlighted the need for accurate, standardised pathological assessment and classification. Recent guidelines have gone some way to set standards and, at a tissue level, our understanding of patterns of tumours response has increased. Nevertheless, work still needs to be done and there is the potential to refine and improve current systems. Future research comparing histological and imaging biomarkers of response to neoadjuvant systemic therapies will also be crucial to improving patient management, for example for future trials omitting surgery for patients with complete response to NACT or, conversely, identifying those with limited tumour response who may benefit from additional systemic therapy prior to surgery.

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Table 1 Residual Cancer Burden Categories

	Complete Pathological Response (pCR)	RCB I	RCB II	RCB 3
Amount of residual tumour present (Calculation of: tumour in mm in two dimensions; % cellularity of the carcinoma; % of DCIS; size of largest nodal metastasis; number of nodes with metastasis)	None	Minimal	Moderate	Extensive
Numerical cut -off	0	<1.36	1.36-3.28	>3.28
Risk of distant recurrence		Low	Intermediate	High

Figure Legends

Figure 1: Low power magnification (haematoxylin and eosin stained section) of a portion of

wide local excision post-neoadjuvant chemotherapy showing an area of fibrosis (lower portion of tissue) representing the tumour bed (site of tumour before treatment). The solid arrow shows the site at which a marker coil had previously been removed in the laboratory prior to sampling and processing (shown at higher magnification in **Figure 2**) and the dashed arrow shows the site of small clusters of residual invasive carcinoma (not visible at this power but shown at a higher magnification in **Figure 3**).

Figure 2: Confirmation of the presence of tumour bed around an inserted marker. The tumour bed is seen as fibrous stroma. This is focally loose and oedematous but elsewhere is more hyalinised. The site of marker is seen as a gap in the tissue (bottom left). No residual cancer cells are seen in this area.

Figure 3: Residual invasive breast cancer cells and stromal calcification post-neoadjuvant chemotherapy. Small clusters of residual invasive cancer cells (arrows) are seen, and an area of microcalcification in the stroma adjacent to an uninvolved residual duct.

Figure 1

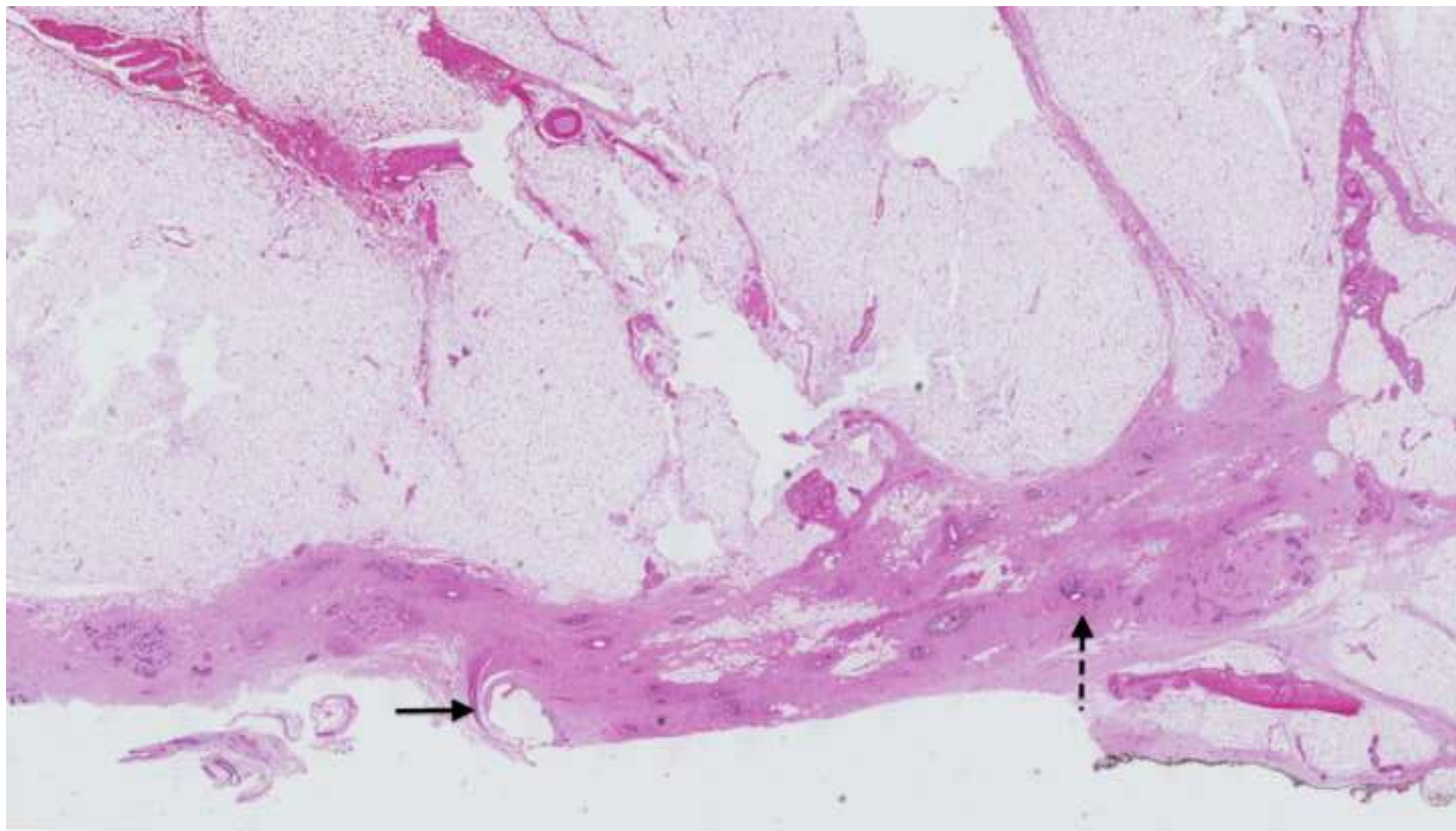


Figure 2

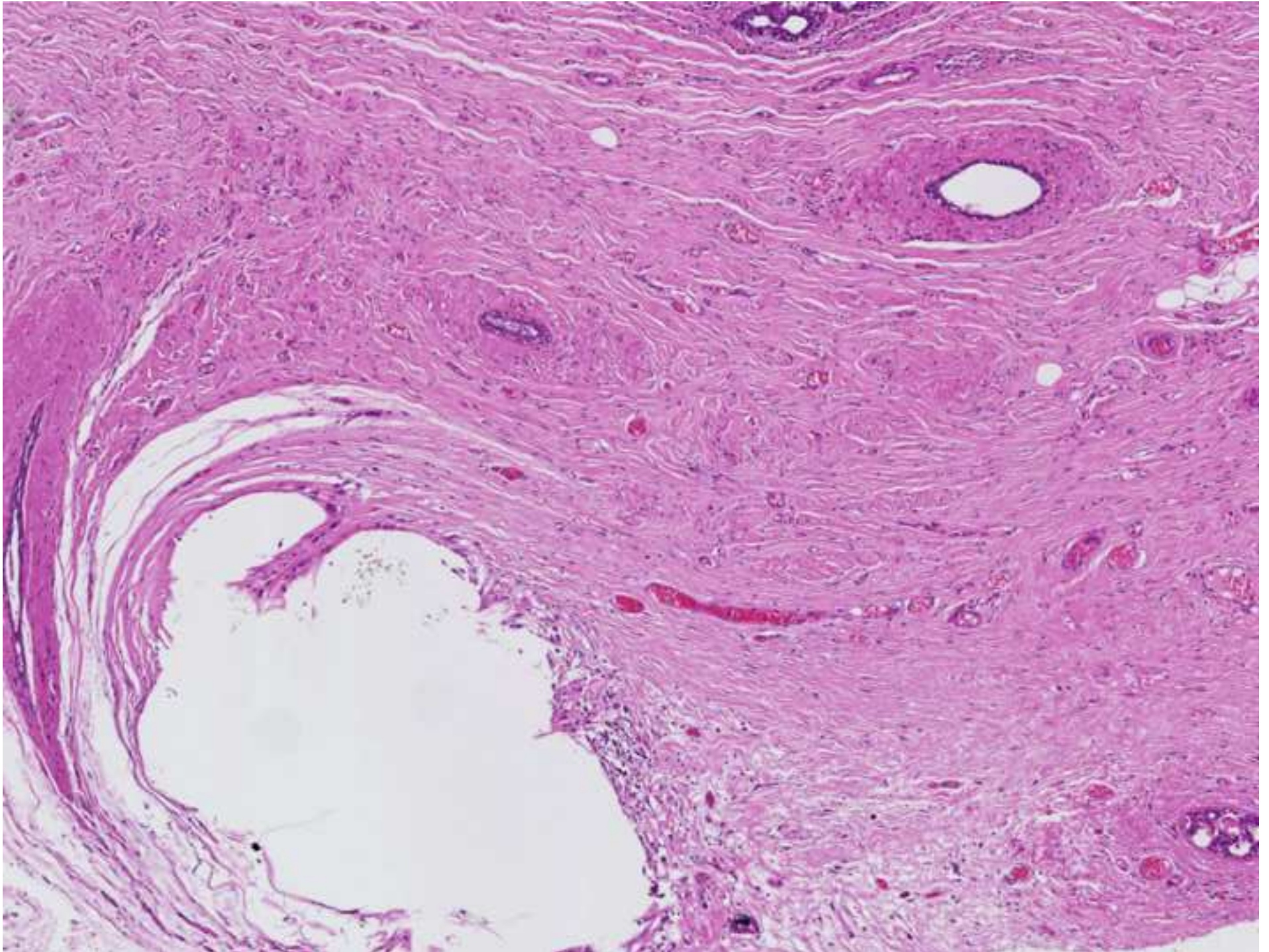


Figure 3

[Click here to download Figure Fig 3 NACT calc and residual tumour cells.tif](#)

